MoFlo™ Astrios™ Enhanced Forward Scatter: Simultaneous Sorting of Micro- and Macro- samples

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Introduction
One of the major features of the MoFlo™ Astrios™ system is a forward scatter module that allows the simultaneous collection of two independent forward scatters. Furthermore, small particle resolution has been improved to allow the resolution of 200 nm beads from instrument noise and the resolution of 300 and 300 nm beads from each other. Simultaneously, this second forward scatter parameter can be set to study large particles, seven different forward scatter modules allow unique combinations to be explored and novel populations to be discovered.

This poster examines minimally prepared whole blood samples to be studied and sorted. The goal of the exercise is to understand the difference between freshly collected and stabilized blood, and to develop a standardized protocol for verifying the wide range of Astrios™ features using cellular samples.

Materials
Fresh whole blood collected in yellow top tube (BD, 364000). Immuno-TROL™ (Beckman Coulter 600507F1, CD45-Pacific Blue (Beckman Coulter #74765, 485-496/564), CD45-PC7 (Beckman Coulter #74765, 531-543/600), CD25-5C1 (Beckman Coulter #74765, 485-496/564). Reference 200, 300, and 1000 nanometer polystyrene beads (Bangs Laboratories #70105, ATTO N, FITC N, ATTO 488, 540, 647, Ultra Rainbow Calibration Beads (CytoMics 121540, CytoSoft 121540), Surflon Sheath (Beckman Coulter 841440), and sterile filtered (0.2 µm) phosphate buffered saline (PBS).

Methods
A combination of three antibodies conjugated (CD45-Pacific Blue, CD-45-PE, and CD-35-PC7). 30 µl each was added to 100 µl of whole blood (either fresh or stabilized) and incubated for 15 minutes at room temperature in the dark. Following the incubation 4 ml of sterile filtered PBS was added to the tubes. A relative size reference 1000 nm beads were added to the preparation.

Instrument Set-up
To minimize background sheath and air contamination the Astrios™ was equipped with a 0.94 µm inline sheath filter and 0.2 µm air filter.

Flow Cytometry
The Astrios™ was set to trigger on the 644nm laser side scatter parameter and threshold was set to allow 300 000-500000 events (instrument, fluids, and optical noise). Sheath and sample pressure were set to allow the sample to run at approximately 50 000-60 000 psi. For sorting, the stream frequency, amplitude, voltage, and data delay were set with identical data and sorted in parallel with a set of 300 000 events. The delta and pulse width were optimized to allow the reuse of the sample and the high event rate the efficiency rate ranged from 50 to 60% during the sort.

Analysis and Sorting Strategy
To eliminate the vast number of erythrocytes from the analysis, cells negative for CD255 was selected. These cells were further selected using CD45 to study lymphocyte, monocyte, and granulocyte populations (green path) or CD45 to examine platelets and other uncharacterized CD45-negative populations (yellow path). These populations were further evaluated using the CD45-negative population and four other forward scatter parameters, which may represent various subpopulations of the population. The sorting of subpopulations is based on the forward scatter parameter (blue path). It was important to understand and mark these populations through sorting and analysis and sorting.

Discussion
We were able to successfully create a minimalist assay allowing the analysis of both large and small population present in whole blood. The unique features of the Astrios™ allowed for the high speed analysis and sorting of these populations. Whole immuno-TROL™ populations are not as “leaky” compared to freshly drawn blood, the exercise demonstrates that these cells can be used as a standard to set up and test the system. This standard method can be used in sorting and characterizing various subpopulation in whole blood using the advanced optics of the Astrios™ system.

Conclusions
The Astrios™ systems enhanced Forward Scatter module (patent pending) enables users to visualize and sort subpopulation in whole blood simultaneously.

The method presented in this poster demonstrates a standard process for preparing and testing the Astrios™ system for studies of widely disparate populations in whole Blood.

Forward Scatter modes used on the Astrios™ generate population user the opportunity to enhance the separation and characterization of various populations based on material composition, size, and morphology in different sample types. See poster B108 for further examples of the expanding use of the Astrios™ system.

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* For Research Purposes only, not for diagnostic purposes.

Product not yet released.